(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/18209 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/50, 16/22, G01N 33/53, A61K 38/18, 31/70, 39/395, A01K 67/027

(21) International Application Number: PCT/US00/24863

(22) International Filing Date:
8 September 2000 (08.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/153,303	10 September 1999 (10.09.1999)	US
60/194,246	3 April 2000 (03.04.2000)	US
09/619,251	19 July 2000 (19.07.2000)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/153,303 (CIP)
Filed on	10 September 1999 (10.09.1999)
US	60/194,246 (CIP)
Filed on	3 April 2000 (03.04.2000)
US	09/619,251 (CIP)
Filed on	19 July 2000 (19.07.2000)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/18209 A1

(54) Title: FIBROBLAST GROWTH FACTOR POLYPEPTIDE AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: The present invention provides FGF19X, a novel isolated polypeptide, as well as a polynucleotide encoding FGF19X and antibodies that immunospecifically bind to FGF19X or any derivative, variant, mutant, or fragment of the FGF19X polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the FGF19X polypeptide, polynucleotide and antibody are used in detection and treatment of a broad range of pathological states, as well as to other uses.

FIBROBLAST GROWTH FACTOR POLYPEPTIDE AND NUCLEIC ACIDS ENCODING SAME

FIELD OF THE INVENTION

This invention relates to polypeptide growth factors to polynucleotides encoding them, and to methods of using them in therapeutic and diagnostic applications. More specifically, the polypeptides and polynucleotides are related to fibroblast growth factor-19 (FGF-19).

BACKGROUND OF THE INVENTION

The fibroblast growth factor (FGF) family consists of a number of structurally related polypeptide growth factors that are heparin-binding polypeptides. Their expression is controlled at the levels of transcription, mRNA stability, and translation. The bioavailability of FGFs is further modulated by post-translational processing and regulated protein trafficking. FGFs typically bind to receptor tyrosine kinases (fibroblast growth factor receptors, FGFRs), heparan sulfate proteoglycans (HSPG), and a cysteine-rich FGF receptor (CFR).

FGF signaling is mediated by a dual-receptor system consisting of (*i*) four high-affinity tyrosine kinase receptors, termed fibroblast growth factor receptors (FGFRs), and (*ii*) low-affinity heparan sulfate proteoglycan receptors that enhance ligand presentation to the FGFRs. FGFRs are required for most biological activities of FGFs. HSPGs alter FGF:FGFR interactions and CFR participates in FGF intracellular transport. FGF signaling pathways are intricate and are intertwined with insulin-like growth factor, transforming growth factor-beta, bone morphogenetic protein, and vertebrate homologs of Drosophila wingless activated pathways. These molecules have been implicated in a variety of human neoplasms.

FGFs are major regulators of embryonic development. They influence the formation of the primary body axis, neural axis, limbs, and other structures. The activities of FGFs depend on their coordination of fundamental cellular functions, such as survival, replication, differentiation, adhesion, and motility, through effects on gene expression and the cytoskeleton. Several FGFs, including FGF-1, -2, -3, -4, -5, -6, and -7, and several FGF variants, play important roles in the pathobiology of many diseases. Thus, FGFs are target candidates for many pharmaceuticals currently being developed. A need remains to identify all remaining novel FGF family members and characterize their roles in the onset and progression

of diseases. A need also remains to develop therapeutics that target the novel FGFs so as to treat or prevent their associated diseases.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the discovery of a nucleic acid encoding a novel polypeptide having homology to Fibroblast Growth Factor-19 (FGF-19) protein. Fibroblast Grown Factor-19X (FGF19X) polynucleotide sequences and the FGF19X polypeptides encoded by these nucleic acid sequences, and fragments, homologs, analogs, and derivatives thereof, are claimed in the invention.

In one aspect, the invention provides an isolated FGF19X nucleic acid (SEQ ID NO:1, as shown in Table 1), that encodes a FGF19X polypeptide, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, *e.g.*, nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide comprising the amino acid sequence of Table 1 (SEQ ID NO:2). The nucleic acid can be, *e.g.*, a genomic DNA fragment, or it can be a cDNA molecule. In another aspect, the invention provides a complement to the FGF19X nucleic acid shown in Table 1, or a fragment, homolog, analog or derivative thereof.

Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

The present invention is also directed to host cells transformed with a recombinant expression vector comprising any of the nucleic acid molecules described above.

In one aspect, the invention includes a pharmaceutical composition that includes a FGF19X nucleic acid and a pharmaceutically acceptable carrier or diluent. In a further aspect, the invention includes a substantially purified FGF19X polypeptide, *e.g.*, any of the FGF19X polypeptides encoded by a FGF19X nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition that includes a FGF19X polypeptide and a pharmaceutically acceptable carrier or diluent.

In a further aspect, the invention provides an antibody that binds specifically to a FGF19X polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including FGF19X antibody and a pharmaceutically acceptable carrier or diluent. The present invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

The present invention is further directed to kits comprising antibodies that bind to a polypeptide encoded by any of the nucleic acid molecules described above and a negative control antibody.

The invention further provides a method for producing a FGF19X polypeptide. The method includes providing a cell containing a FGF19X nucleic acid, *e.g.*, a vector that includes a FGF19X nucleic acid, and culturing the cell under conditions sufficient to express the FGF19X polypeptide encoded by the nucleic acid. The expressed FGF19X polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous FGF19X polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

The present invention provides a method of inducing an immune response in a mammal against a polypeptide encoded by any of the nucleic acid molecules disclosed above. In one embodiment, the method includes administering to the mammal an amount of the polypeptide sufficient to induce the immune response. In another embodiment, the method includes administering to the mammal a nucleic acid encoding a FGF19X polypeptide in an amount sufficient to produce enough FGF19X polypeptide to induce the immune response.

The present invention is also directed to methods of identifying a compound that binds to FGF19X polypeptide by contacting the FGF19X polypeptide with a compound and determining whether the compound binds to the FGF19X polypeptide.

The invention further provides methods of identifying a compound that modulates the activity of a FGF19X polypeptide by contacting FGF19X polypeptide with a compound and determining whether the FGF19X polypeptide activity is modified.

The present invention is also directed to compounds that modulate FGF19X polypeptide activity identified by contacting a FGF19X polypeptide with the compound and determining whether the compound modifies activity of the FGF19X polypeptide, binds to the FGF19X polypeptide, or binds to a nucleic acid molecule encoding a FGF19X polypeptide.

In another aspect, the invention provides a method of diagnosing a tissue proliferation-associated disorder, such as tumors, restenosis, psoriasis, diabetic and post-surgery complications, and rheumatoid arthritis, in a subject. The potential role(s) of FGF19X in tumorigenesis may include autocrine stimulation of tumor growth, hormone independence, angiogenesis, metastatic progression, chemoresistance, radiotherapy resistance, survival in trophic factor limited secondary tissue site microenvironments, and stimulation of tumor cell matrix degradation and tumor cell migration (*i.e.*, tumor invasion). The method includes providing a protein sample from the subject and measuring the amount of FGF19X polypeptide in the subject sample. The amount of FGF19X in the subject sample is then

compared to the amount of FGF19X polypeptide in a control protein sample. An alteration in the amount of FGF19X polypeptide in the subject protein sample relative to the amount of FGF19X polypeptide in the control protein sample indicates the subject has a tissue proliferation-associated condition. A control sample is preferably taken from a matched individual, *i.e.*, an individual of similar age, sex, or other general condition but who is not suspected of having a tissue proliferation-associated condition. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a tissue proliferation-associated disorder. In some embodiments, the FGF19X polypeptide is detected using a FGF19X antibody.

In a further aspect, the invention includes a method of diagnosing a tissue proliferation-associated disorder, such as tumors, restenosis, psoriasis, diabetic and post-surgery complications, and rheumatoid arthritis, in a subject. The method includes providing a nucleic acid sample, *e.g.*, RNA or DNA, or both, from the subject and measuring the amount of the FGF19X nucleic acid in the subject nucleic acid sample. The amount of FGF19X nucleic acid sample in the subject nucleic acid is then compared to the amount of FGF19X nucleic acid in a control sample. An alteration in the amount of FGF19X nucleic acid in the sample relative to the amount of FGF19X in the control sample indicates the subject has a tissue proliferation-associated disorder.

In a further aspect, the invention includes a method of diagnosing a tissue proliferation-associated disorder in a subject. The method includes providing a nucleic acid sample from the subject and identifying at least a portion of the nucleotide sequence of a FGF19X nucleic acid in the subject nucleic acid sample. The FGF19X nucleotide sequence of the subject sample is then compared to a FGF19X nucleotide sequence of a control sample. An alteration in the FGF19X nucleotide sequence in the sample relative to the FGF19X nucleotide sequence in said control sample indicates the subject has a tissue proliferation-associated disorder.

In a still further aspect, the invention provides method of treating or preventing or delaying a tissue proliferation-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired a FGF19X nucleic acid, a FGF19X polypeptide, or a FGF19X antibody in an amount sufficient to treat, prevent, or delay a tissue proliferation-associated disorder in the subject.

The tissue proliferation-associated disorders diagnosed, treated, prevented or delayed using the FGF19X nucleic acid molecules, polypeptides or antibodies can involve epithelial cells, *e.g.*, fibroblasts and keratinocytes in the anterior eye after surgery. Other tissue

proliferation-associated disorder include, *e.g.*, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi sarcoma, and rheumatoid arthritis.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a representation of hFGF19-X protein expressed in *E. coli* cells, wherein the two lanes show the FGF19X protein from two independent isolates.

FIG. 2 is a representation of hFGF19-X protein secreted by 293 cells.

DETAILED DESCRIPTION OF THE INVENTION

Fibroblast growth factors (FGFs) are a family of growth factors and oncogene products that mediate diverse cellular responses during embryonic, fetal, and postnatal vertebrate development. FGFs also play important roles in multiple physiologic functions, including angiogenesis, mitogenesis, pattern formation, morphogenesis, cellular growth and differentiation, tissue repair, inflammation, metabolic regulation, oncogenesis and metastatic progression. See, *e.g.*, Goldfarb 1996 *Cytokine Growth Factor Rev* 7: 311-325. The FGFs contain a conserved core of approximately 120 amino acid residues. Members of the fibroblast growth factor family, with its prototype members acidic FGF (FGF-1) and basic FGF (FGF-2), bind to four related receptor tyrosine kinases, expressed on most types of cells in tissue culture. Although fibroblast growth factor tyrosine kinase receptors are encoded by four genes, alternate splicing can result in more than 100 possible protein receptor sequences.

The stimulation of cellular metabolism by FGFs is mediated by a dual-receptor system. See, *e.g.*, Fernig *et al.* 1994 *Prog Growth Factor Res* 5: 353-377. This system includes a family of four receptor tyrosine kinases (FGFR) and the heparan sulphate proteoglycans

(HSPG). The stimulation of cell division by FGFs requires both partners of the dual-receptor system to be present. The binding of FGFs to the FGFRs is marked by a pattern of overlapping specificity despite alternative splicing events generating a large number of FGFR proteins. Thus many of the FGFR isoforms bind several FGFs. It is likely that each FGF requires a different pattern of sulphation within the heparan sulphate chains for binding. Therefore, the HSPG receptors may provide additional specificity, allowing a cell to fine tune its response to the FGFs present in the extracellular milieu. The HSPG receptors also control the availability of FGFs and hence regulate the transport of FGFs within a tissue. FGF-stimulated cell division appears to require that the FGFs be translocated to the nucleus via the cytosol after interacting with the dual-receptor system. *In vivo* data demonstrate that FGF receptors and their ligands are targets for anti-cancer therapy development. Given the complexity of this family, it is possible that a unique targetable FGF receptor or FGF isoform can be found in one or more tumor types.

FGF19, also known as the heparin dependent ligand for FGFR4, was identified as a human expressed sequence tag (EST) that encodes a protein similar to mouse FGF15. See, *e.g.*, Nishimura *et al.* 1999 *Biochim. Biophys. Acta* 1444: 148-151. The human fetal brain cDNAs corresponding to the complete coding region of FGF19 was isolated, and the deduced 216 amino acid FGF19 protein was shown to contain a signal sequence and two cysteine residues that are conserved in the FGF family. See, Nishimura *et al.* 1999, above. Human FGF19 and mouse FGF15 share approximately 51% amino acid identity, suggesting that the two proteins belong to the same FGF subfamily. See, Nishimura *et al.* 1999, above. PCR analysis of cDNAs derived from human adult tissues and human fetal brain showed that FGF-19 is expressed in fetal brain, suggesting that FGF19 is involved in brain development during embryogenesis. See, Nishimura *et al.* 1999 *Biochim. Biophys. Acta* 1444: 148-151. FGF-19 is predominately expressed in fetal brain, skin, cartilage and retina, gall bladder. Available evidence indicates that FGF-19 potentially plays an important role in brain development. Most notably, FGF-19 is overexpressed in colon adenocarcinoma cell lines. See, Nishimura *et al.*, 1999, above.

The invention is based in part on the discovery of novel nucleic acid sequences encoding polypeptides similar to growth factors, and more specifically, to human fibroblast growth factor FGF-19. The novel nucleic acids described herein and their encoded polypeptides are described in detail below.

FGF19X: Fibroblast Growth Factor-19 Homolog

Included in the invention is a nucleic acid encoding a novel fibroblast growth factor (FGF19X, also referred to as AC009002-A). Anitbodies that bind specifically to FGF19X polypeptides, or fragments thereof, are also included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of FGF19X nucleic acids, polypeptides, and antibodies.

FGF19X, a novel FGF-19 homolog, was identified from human genomic DNA. TABLE 1 depicts the FGF19X polynucleotide (SEQ ID NO:1) and translated polypeptide (SEQ ID NO:2) sequences of the invention. Of the 514 nucleotides of FGF19X, nucleotides 52 to 510 encode a 153 amino acid polypeptide that is highly homologous to the carboxy-terminus of human FGF-19. An initiation codon is detected at positions 52-54, and a TAG stop codon is found at 511-513. The predicted molecular weight of the protein is 16754.7 Da.

TABLE 1
FGF19X Polynucleotide (SEQ ID NO:1) and Amino Acid Sequences (SEQ ID NO:2)
(Translated Protein - Frame: 1 - Nucleotide 52 to 510)

```
   1 CAGCTTCTCCGAGCTCACACCCCGGAGATCACCTGAGGACCCGAG

  46 CCATTGATGGACTCGGACGAGACCGGGTTCGAGCACTCAGGACTG
           MetAspSerAspGluThrGlyPheGluHisSerGlyLeu

  91 TGGGTTTCTGTGCTGGCTGGTCTTCTGCTGGGAGCCTGCCAGGCA
     TrpValSerValLeuAlaGlyLeuLeuLeuGlyAlaCysGlnAla

 136 CACCCCATCCCTGACTCCAGTCCTCTCCTGCAATTCGGGGGCCAA
     HisProIleProAspSerSerProLeuLeuGlnPheGlyGlyGln

 181 GTCCGGCAGCGGTACCTCTACACAGATGATGCCCAGCAGACAGAA
     ValArgGlnArgTyrLeuTyrThrAspAspAlaGlnGlnThrGlu

 226 GCCCACCTGGAGATCAGGGAGGATGGGACGGTGGGGGGCGCTGCT
     AlaHisLeuGluIleArgGluAspGlyThrValGlyGlyAlaAla

 271 GACCAGAGCCCCGAAAGTCTCCTGCAGCTGAAAGCCTTGAAGCCG
     AspGlnSerProGluSerLeuLeuGlnLeuLysAlaLeuLysPro

 316 GGAGTTATTCAAATCTTGGGAGTCAAGACATCCAGGTTCCTGTGC
     GlyValIleGlnIleLeuGlyValLysThrSerArgPheLeuCys

 361 CAGCGGCCAGATGGGGCCCTGTATGGATCGCTCCACTTTGACCCT
     GlnArgProAspGlyAlaLeuTyrGlySerLeuHisPheAspPro

 406 GAGGCCTGCAGCTTCCGGGAGCTGCTTCTTGAGGACGGATACAAT
     GluAlaCysSerPheArgGluLeuLeuLeuGluAspGlyTyrAsn

 451 GTTTACCAGTCCGAAGCCCACGGCCTCCCGCTGCACCTGCCAGGG
     ValTyrGlnSerGluAlaHisGlyLeuProLeuHisLeuProGly

 496 TTACAGAGGAGGCTCTAGA
     LeuGlnArgArgLeu
```

FGF19X polypeptide has a signal peptide at its amino terminal end, with a predicted cleavage site between positions 28 and 29 (*i.e.*, at the dash in the sequence CysGlnAla-HisPro). Thus, the invention includes alternative forms of FGF19X either with and without the signal peptide from amino acid positions 1 through 28. For example, in separate embodiments, the invention includes a polypeptide comprising amino acids 28-153 of SEQ ID NO:2, or a polypeptide comprising amino acids 1-153 of SEQ ID NO:2. In a further embodiment, the invention provides a polypeptide comprising amino acids 1-28 of SEQ ID NO:2, or a region of amino acids 1-28 that is sufficient to direct a linked polypeptide sequence to a desired cellular location. The invention also includes fragments, homologs, analogs, and derivatives of FGF19X nucleic acids and polypeptides.

TABLE 2 depicts an alignment of the regions of the amino acid sequence of FGF19X with regions of human FGF-19 polypeptide sequence (SEQ ID NO:3). As denoted in Table 2 and throughout the specification, "identical" residues correspond to those residues in a comparison between two sequences where the equivalent nucleotide base or amino acid residues in an alignment of two sequences are the same. Residues are "positive" when the comparisons between two sequences in an alignment show that residues in an equivalent position in a comparison are either the same amino acid or a conserved amino acid as defined below. Identical amino acids are denoted in the alignment by "|". Conserved amino acids that are positive, but not identical, are denoted in the alignment by "+". See the section below entitled DETERMINING HOMOLOGY BETWEEN TWO OR MORE SEQUENCES for further discussion of "percent of amino acid identity" and "percent of positive amino acids."

TABLE 2: BLASTP Results Showing FGF19X Homology To FGF19

Query = FGF19X (SEQ ID NO:2); Sbjct = Human FGF-19 (SEQ ID NO:3)

```
ptnr:TREMBLNEW-ACC:AAD45973 FIBROBLAST GROWTH FACTOR 19 - HOMO SAPIENS (HUMAN), 216 aa.
Length = 216
Score = 236 (83.1 bits), Expect = 6.6e-20, P = 6.6e-20
Identities = 58/142 (40%), Positives = 80/142 (56%)

Query:    15 VSVLAGLLLGACQAHPIP--DSSPLLQFG-GQ-VRQRYLYTDDAQQ-TEAHLEIREDGTV 69
             | +|||| | |     |+    |+ | + +| |  +| |+|||       +    | || || |
Sbjct:    10 VWILAGLWL-AVAGRPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVV 68

Query:    70 GGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLE 129
               |  ||  |||++||+    + | || + |+||   || + | | +  | |+| | +
Sbjct:    69 DCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEEEIRP 128

Query:   130 DGYNVYQSEAHGLPLHLPGLQRR 152
             |||||||||  | ||+ |    ++|
Sbjct:   129 DGYNVYRSEKHRLPVSLSSAKQR 151
```

The full amino acid sequence of the FGF19X protein has 58 of 142 amino acid residues (40%) identical to, and 80 of 142 residues (56%) positive with, the 216 amino acid residue human fibroblast growth factor 19 protein (SPTREMBL accession number AAD45973), as shown in Table 2. In additional searches of sequence databases, it was found, for example, that the FGF19X nucleic acid sequence has 334 of 380 bases (87 %) identical to a partial coding sequence of a human gene for alpha 1,2-fucosyltransferase, (GENBANK accession number AB006136). BLASTX analysis also showed that 58 of 142 residues (40%) are identical to, and 80 of 142 residues (56%) are positive with the 216 residue human FGF-8 homolog PRO533. See, *e.g.*, PCT Publication WO 99/14327).

The potential role(s) of FGF19X in tumorigenesis may include autocrine stimulation of tumor growth, hormone independence, angiogenesis, metastatic progression, chemoresistance, radiotherapy resistance, survival in trophic factor limited secondary tissue site microenvironments, and stimulation of tumor cell matrix degradation and tumor cell migration (*i.e.*, tumor invasion).

FGF19X is preferentially expressed in a restricted subset of cancer tissues, namely, small cell lung cancer and colon adenocarcinoma. See EXAMPLE 5, below. Based on this result, successful targeting of FGF19X using a monoclonal antibody should have an inhibitory effect on tumor growth, matrix invasion, chemoresistance, radio-resistance, metastatic dissemination, or any combination thereof. In addition, neutralization of FGF19X-encoded protein is predicted to block or limit the extent of tumor neovascularization.

FGF19X Nucleic Acids

TABLE 3 below summarizes the disclosed FGF19X nucleic acid sequences, encoded polypeptides, and the corresponding sequence identifier numbers (SEQ ID NOs) for the various disclosed sequences and clones containing these nucleic acids and polypeptides.

TABLE 3: Summary Of Nucleic Acids And Proteins Of The Invention

SEQUENCE NAME	LOCATION	NUCLEIC ACID SEQ ID NOs	POLYPEPTIDE SEQ ID NOs
FGF19X	TABLE 1	SEQ ID NO:1	SEQ ID NO:2
FGF19	TABLE 2		SEQ ID NO:3
FGF19X MAT Forward	Example 1	SEQ ID NO:4	
FGF19X MAT Reverse	Example 1	SEQ ID NO:5	
pSec-V5-His Forward	Example 2	SEQ ID NO:6	
pSec-V5-His Reverse	Example 2	SEQ ID NO:7	
Linker 1	Example 3	SEQ ID NO:8	
Linker 2	Example 3	SEQ ID NO:9	

Ab78 (F)	Example 4	SEQ ID NO:10	
Ab78 (R)	Example 4	SEQ ID NO:11	
Ab78 (P)	Example 4	SEQ ID NO:12	

The novel nucleic acids of the invention include those that encode a FGF19X or FGF19X-like protein. Among these nucleic acids is the nucleic acid whose sequence is provided in SEQ ID NO:1, or a fragment thereof. Additionally, the invention includes mutant or variant nucleic acids of SEQ ID NO:1, or a fragment thereof, any of whose bases may be changed from the corresponding base shown in SEQ ID NO:1 while still encoding a protein that maintains its FGF19X-like activities and physiological functions. The invention further includes the complement of the nucleic acid sequence of SEQ ID NOs:1, including fragments, derivatives, analogs and homolog thereof. Examples of the complementary strand of portions of FGF19X are shown as oligonucleotide primers in the EXAMPLES section. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

One aspect of the invention pertains to isolated nucleic acid molecules that encode FGF19X proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify FGF19X-encoding nucleic acids (*e.g.*, FGF19X mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of FGF19X nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 20 nt, 30 nt, 50 nt, 100 nt, 500 nt, 1000 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules.

Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated FGF19X nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, 0.1 kb or 0.01 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or a complement of any of this nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 as a hybridization probe, FGF19X nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to FGF19X nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at lease 6 contiguous nucleotides of SEQ ID NO:1, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO:1, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, *e.g.*, a fragment that can be used as a probe or primer, or a fragment encoding a biologically active portion of FGF19X. "Fragments" provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. "Derivatives" are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. "Analogs" are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or

analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, 85%, 90%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of FGF19X polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a FGF19X polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human FGF19X protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:2, as well as a polypeptide having FGF19X activity. Biological activities of the FGF19X proteins are described below. A homologous amino acid sequence does not encode the amino acid sequence of a human FGF19X polypeptide.

The nucleotide sequence determined from the cloning of the human FGF19X gene allows for the generation of probes and primers designed for use in identifying and/or cloning FGF19X homologues in other cell types, *e.g.*, from other tissues, as well as FGF19X

homologues from other mammals. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NO:1; or an anti-sense strand nucleotide sequence of SEQ ID NO:1; or of a naturally occurring mutant of SEQ ID NO:1.

Probes based on the human FGF19X nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a FGF19X protein, such as by measuring a level of a FGF19X-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting FGF19X mRNA levels or determining whether a genomic FGF19X gene has been mutated or deleted.

A "polypeptide having a biologically active portion of FGF19X" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of FGF19X" can be prepared by isolating a portion of SEQ ID NO:1 that encodes a polypeptide having a FGF19X biological activity (biological activities of the FGF19X proteins are described below), expressing the encoded portion of FGF19X protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of FGF19X. For example, a nucleic acid fragment encoding a biologically active portion of FGF19X can optionally include an ATP-binding domain. In another embodiment, a nucleic acid fragment encoding a biologically active portion of FGF19X includes one or more regions.

FGF19X variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO:1 due to degeneracy of the genetic code. These nucleic acids thus encode the same FGF19X protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

In addition to the human FGF19X nucleotide sequence shown in SEQ ID NO:1, those skilled in the art will appreciate that DNA sequence polymorphisms that lead to changes in the

amino acid sequences of FGF19X may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the FGF19X gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a FGF19X protein, preferably a mammalian FGF19X protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the FGF19X gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in FGF19X that are the result of natural allelic variation and that do not alter the functional activity of FGF19X are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FGF19X proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO:1 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FGF19X cDNAs of the invention can be isolated based on their homology to the human FGF19X nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. In one example, a soluble human FGF19X cDNA can be isolated based on its homology to human membrane-bound FGF19X. Likewise, a membrane-bound human FGF19X cDNA can be isolated based on its homology to soluble human FGF19X. In another example, a splice variant of a FGF19X cDNA can be isolated based on its homology to presently disclosed FGF19X nucleic acids.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding FGF19X proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no

other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA 78*: 6789-6792.

Conservative mutations

In addition to naturally-occurring allelic variants of the FGF19X sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, thereby leading to changes in the amino acid sequence of the encoded FGF19X protein, without altering the functional ability of the FGF19X protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of FGF19X without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the FGF19X proteins of the present invention, are predicted to be particularly unamenable to alteration.

Another embodiment pertains to nucleic acid molecules encoding FGF19X proteins that contain changes in amino acid residues that are not essential for activity. Such FGF19X proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO:2. Preferably, the protein encoded by the nucleic acid is at least about 80% homologous to SEQ ID NO:2, more preferably at least about 85%, 90%, 95%, 98%, and most preferably at least about 99% homologous to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a FGF19X protein homologous to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NO:1 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in FGF19X is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a FGF19X coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for FGF19X biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substitued amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY.

In one embodiment, a mutant FGF19X protein can be assayed for (1) the ability to form protein:protein interactions with other FGF19X proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant FGF19X protein and a FGF19X receptor; (3) the ability of a mutant FGF19X protein to bind to an intracellular

target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (4) the ability to bind BRA protein; or (5) the ability to specifically bind an anti-FGF19X protein antibody.

Antisense

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FGF19X coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a FGF19X protein of SEQ ID NO:2, or antisense nucleic acids complementary to a FGF19X nucleic acid sequence of SEQ ID NO:1 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding FGF19X. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of human FGF19X corresponds to SEQ ID NO:2). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding FGF19X. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding FGF19X disclosed herein (*e.g.*, SEQ ID NO:1), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of FGF19X mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of FGF19X mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of FGF19X mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological

stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyluracil, 5-carboxymethylaminomethyl-2-thiouridine, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2-methyladenine, 2-methylguanine, 2,2-dimethylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 5-methyl-2-thiouracil, 2-thiocytosine, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a FGF19X protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of

antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Ribozymes and PNA moieties

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave FGF19X mRNA transcripts to thereby inhibit translation of FGF19X mRNA. A ribozyme having specificity for a FGF19X-encoding nucleic acid can be designed based upon the nucleotide sequence of a FGF19X DNA disclosed herein (*i.e.*, SEQ ID NO:1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a FGF19X-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, FGF19X mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, FGF19X gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the FGF19X (*e.g.*, the FGF19X promoter and/or enhancers) to form triple helical structures that prevent transcription of the FGF19X gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of FGF19X can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of FGF19X can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of FGF19X can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup *et al.* 1996, above); or as probes or primers for DNA sequence and hybridization. See, *e.g.*, Hyrup *et al.* 1996, above; Perry-O'Keefe 1996, above.

In another embodiment, PNAs of FGF19X can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of FGF19X can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation. See, *e.g.*, Hyrup 1996, above. The synthesis of PNA-DNA chimeras can be performed by any method known in the art. See, *e.g.*, Hyrup *et al.* 1996 above; Finn *et al.* 1996 *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. See, *e.g.*, Mag *et al.* 1989 *Nucl Acid Res* 17: 5973-88. PNA

monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. See, *e.g.*, Finn *et al.* 1996, above. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, *e.g.*, Petersen *et al.* 1975 *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

FGF19X polypeptides

As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by

way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The novel protein of the invention includes the FGF19X-like protein whose sequence is provided in SEQ ID NO:2. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:2 while still encoding a protein that maintains its FGF19X-like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, an FGF19X -like variant that preserves FGF19X-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated FGF19X proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FGF19X antibodies. In one embodiment, native FGF19X proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, FGF19X proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a FGF19X protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FGF19X protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of FGF19X protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of FGF19X protein having less than about 30% (by dry weight) of non-FGF19X protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FGF19X protein, still more preferably less than about 10% of non-FGF19X protein, and

most preferably less than about 5% non-FGF19X protein. When the FGF19X protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of FGF19X protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of FGF19X protein having less than about 30% (by dry weight) of chemical precursors or non-FGF19X chemicals, or alternatively less than about 20%, less than about 10% or less than about 5% of chemical precursors or non-FGF19X chemicals.

Biologically active portions of a FGF19X protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the FGF19X protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 that include fewer amino acids than the full length FGF19X proteins, and exhibit at least one activity of a FGF19X protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the FGF19X protein. A biologically active portion of a FGF19X protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a FGF19X protein of the present invention may contain at least one of the above-identified domains conserved between the FGF19X proteins. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FGF19X protein.

In an embodiment, the FGF19X protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the FGF19X protein is substantially homologous to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the FGF19X protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2 and retains the functional activity of the FGF19X proteins of SEQ ID NO:2.

Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced

in either of the sequences being compared for optimal alignment between the sequences). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG software program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of positive residues.

Chimeric and fusion proteins

The invention also provides FGF19X chimeric or fusion proteins. As used herein, a FGF19X "chimeric protein" or "fusion protein" comprises a FGF19X polypeptide operatively linked to a non-FGF19X polypeptide. A "FGF19X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to FGF19X, whereas a "non-FGF19X polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FGF19X protein, *e.g.*, a protein that is different from the FGF19X protein and that is derived from the same or a different organism. Within a FGF19X fusion protein the FGF19X polypeptide can correspond to all or a portion of a FGF19X protein. In one embodiment, a FGF19X fusion protein comprises at least one biologically active portion of a FGF19X protein. In another embodiment, a FGF19X fusion protein comprises at least two biologically active portions of a FGF19X protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the FGF19X polypeptide and the non-FGF19X polypeptide are fused in-frame to each other. The non-FGF19X polypeptide can be fused to the N-terminus or C-terminus of the FGF19X polypeptide.

For example, in one embodiment a FGF19X fusion protein comprises a FGF19X polypeptide operably linked to the extracellular domain of a second protein. Such fusion proteins can be further utilized in screening assays for compounds that modulate FGF19X activity (such assays are described in detail below).

In another embodiment, the fusion protein is a GST-FGF19X fusion protein in which the FGF19X sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant FGF19X.

In yet another embodiment, the fusion protein is a FGF19X protein containing a heterologous signal sequence at its N-terminus. For example, the native FGF19X signal sequence (*i.e.*, amino acids 1 to 28 of SEQ ID NO:2) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of FGF19X can be increased through use of a heterologous signal sequence.

In another embodiment, the fusion protein is a FGF19X-immunoglobulin fusion protein in which the FGF19X sequences comprising one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The FGF19X-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a

FGF19X ligand and a FGF19X protein on the surface of a cell, to thereby suppress FGF19X-mediated signal transduction *in vivo*. In one nonlimiting example, a contemplated FGF19X ligand of the invention is the FGF19X receptor. The FGF19X-immunoglobulin fusion proteins can be used to affect the bioavailability of a FGF19X cognate ligand. Inhibition of the FGF19X ligand/FGF19X interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the FGF19X-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-FGF19X antibodies in a subject, to purify FGF19X ligands, and in screening assays to identify molecules that inhibit the interaction of FGF19X with a FGF19X ligand.

A FGF19X chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A FGF19X-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FGF19X protein.

FGF19X agonists and antagonists

The present invention also pertains to variants of the FGF19X proteins that function as either FGF19X agonists (mimetics) or as FGF19X antagonists. Variants of the FGF19X protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the FGF19X protein. An agonist of the FGF19X protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FGF19X protein. An antagonist of the FGF19X protein can inhibit one or more of the activities of the naturally occurring form of the FGF19X protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FGF19X

protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the FGF19X proteins.

Variants of the FGF19X protein that function as either FGF19X agonists (mimetics) or as FGF19X antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the FGF19X protein for FGF19X protein agonist or antagonist activity. In one embodiment, a variegated library of FGF19X variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FGF19X variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential FGF19X sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of FGF19X sequences therein. There are a variety of methods which can be used to produce libraries of potential FGF19X variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FGF19X sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

Polypeptide libraries

In addition, libraries of fragments of the FGF19X protein coding sequence can be used to generate a variegated population of FGF19X fragments for screening and subsequent selection of variants of a FGF19X protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a FGF19X coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the FGF19X protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of FGF19X proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FGF19X variants. See, *e.g.*, Arkin and Yourvan 1992 *PNAS* 89: 7811-7815; Delgrave *et al.* 1993 *Protein Engineering* 6: 327-331.

Anti-FGF19X Antibodies

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, $F_{ab'}$ and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated FGF19X polypeptide, or a portion or fragment thereof, may serve as an antigen and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the FGF19X antigen, using standard techniques for polyclonal and monoclonal antibody preparation. Full-length FGF19X protein can be used. Alternatively, the invention provides antigenic peptide fragments of FGF19X for use as immunogens. The antigenic peptide of FGF19X comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO:2. The antigenic peptide encompasses an epitope of FGF19X such that an antibody raised against the peptide forms a specific immune complex with

FGF19X. The antigenic peptide may comprise at least 6 aa residues, at least 8 aa residues, at least 10 aa residues, at least 15 aa residues, at least 20 aa residues, or at least 30 aa residues. In one embodiment of the invention, the antigenic peptide comprises a polypeptide comprising at least 6 contiguous amino acids of SEQ ID NO:2.

In an embodiment of the invention, epitopes encompassed by the antigenic peptide are regions of FGF19X that are located on the surface of the protein, *e.g.*, hydrophilic regions. A hydrophobicity analysis of the human FGF19X protein sequence indicates that the regions between, for example, amino acids 1 to 10 or between amino acids 63 to 70 of SEQ ID NO:2 are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, FGF19X protein sequence of SEQ ID NO:2, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as FGF19X. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human FGF19X proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a FGF19X protein sequence of SEQ ID NO:2, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a FGF19X protein is facilitated by generation of hybridomas that bind to the fragment of a FGF19X protein possessing such a domain. Antibodies that are specific for one or more domains within a FGF19X protein, *e.g.*, the carboxy-terminal residues specific to FGF19X when compared to

FGF-19 (see, *e.g.*, Table 2), or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof. See, *e.g.*, ANTIBODIES: A LABORATORY MANUAL, Harlow and Lane (ed.) 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference. Some of these antibodies are discussed below.

1. Polyclonal Antibodies

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Many methods of purification of immunoglobulins are known in the art. See, *e.g*, Wilkinson, THE SCIENTIST, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28.

2. Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies directed towards a particular FGF19X protein, or derivatives, fragments, analogs or homologs thereof, may be prepared by any method known in the art. Hybridoma techniques that provide for the production of antibody molecules by continuous cell line culture may be utilized. See, *e.g.*, Kohler & Milstein, 1975 *Nature* 256: 495-497. In other embodiments, techniques include, but are not limited to, the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). In a hybridoma method, a mouse, hamster or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent, *i.e.*, FGF19X or fragments or portions thereof. Alternatively, the lymphocytes can be immunized *in vitro*.

The immunizing agent will typically include the FGF19X protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. See, *e.g.*, Goding, 1986 MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE, Academic Press, pp. 59-103. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

In one embodiment, immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. In another embodiment, immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection (ATCC), Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies, and any method known in the art may be used. See, *e.g.*, Kozbor 1984, J. Immunol. 133:3001; Brodeur *et al.*, 1987 MONOCLONAL ANTIBODY PRODUCTION TECHNIQUES AND APPLICATIONS, Marcel Dekker, Inc., New York, pp. 51-63.

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis. See, *e.g.*, Munson and Pollard, 1980 *Anal. Biochem.* 107: 220. It is an objective, especially important in therapeutic applications of monoclonal antibodies, to identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods known in the art. See, *e.g.*, Goding, 1986. MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE, Academic Press, pp. 59-103. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by any recombinant DNA method known in the art. See, *e.g.*, U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to

genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. See, *e.g.*, U.S. Patent No. 4,816,567; Morrison 1994 *Nature* 368: 812-813. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

3. Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following any method known in the art, including, but not limited to, substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. See, *e.g.*, Jones *et al.*, 1986 *Nature*, 321: 522-525; Riechmann *et al.*, 1988 *Nature*, 332: 323-327; Verhoeyen *et al.*, 1988 *Science*, 239: 1534-1536; U.S. Patent No. 5,225,539. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region

(Fc), typically that of a human immunoglobulin. See, *e.g.*, Jones *et al.*, 1986 above; Riechmann *et al.*, 1988 above; Presta 1992 *Curr. Op. Struct. Biol.*, 2: 593-596.

Additionally, recombinant anti-FGF19X antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application No. 125,023; Better *et al.*1988 *Science* 240:1041-1043; Liu *et al.* 1987 *PNAS* 84:3439-3443; Liu *et al.* 1987 *J Immunol.* 139:3521-3526; Sun *et al.* 1987 *PNAS* 84:214-218; Nishimura *et al.* 1987 *Cancer Res* 47:999-1005; Wood *et al.* 1985 *Nature* 314:446-449; Shaw *et al.* 1988, *J. Natl Cancer Inst* 80:1553-1559; Morrison 1985 *Science* 229:1202-1207; Oi *et al.* 1986 *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* 1986 *Nature* 321:552-525; Verhoeyan *et al.* 1988 *Science* 239:1534; and Beidler *et al.* 1988 *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference.

4. Human Antibodies

Fully human antibodies essentially relate to antibody molecules in which the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared and utilized by method known in the art, including, but not limited to, the trioma technique; the human B-cell hybridoma technique, the EBV hybridoma technique; the human hybridoma technique or by transforming human B-cells with Epstein Barr Virus *in vitro*. See, *e.g.*, Kozbor, *et al.*, 1983 *Immunol Today* 4: 72; Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96; Cote, *et al.*, 1983. Proc Natl Acad Sci USA 80: 2026-2030.

In addition, human antibodies can also be produced using additional techniques, including but not limited to, phage display libraries. See, *e.g.*, Hoogenboom and Winter, *1991 J. Mol. Biol.*, 227: 381; Marks *et al.*, 1991 *J. Mol. Biol.*, 222: 581. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. See,

e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016; and in Marks *et al.* 1992 *BioTechnology* 10: 779-783; Lonberg *et al.*, 1994 *Nature* 368: 856-859; Morrison 1994 *Nature* 368: 812-13; Fishwild *et al.*, 1996 *Nature Biotech.* 14: 845-51; Neuberger 1996 *Nature Biotech* 14: 826; Lonberg and Huszar 1995 *Intern. Rev. Immunol.* 13: 65-93.

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. See, *e.g.*, PCT publication WO94/02602. The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. One embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain. See, *e.g.*, U.S. Patent No. 5,939,598. It can be obtained by any method known in the art, including, but not limited to, deleting the "J" segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

Methods for producing an antibody of interest, such as a human antibody. See, *e.g.*, U.S. Patent No. 5,916,771. It also includes introducing an expression vector that contains a

nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity. See, *e.g.*, PCT publication WO 99/53049.

5. F_{ab} Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention. See *e.g.*, U.S. Patent No. 4,946,778. In addition, methods can be adapted for the construction of F_{ab} expression libraries to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. See *e.g.*, Huse *et al.*, 1989 *Science* 246: 1275-1281. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

6. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities. See, *e.g.*, Milstein and Cuello 1983 *Nature* 305: 537-539. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by

affinity chromatography steps. See, *e.g.*, WO93/08829; Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. In one embodiment, the fusion is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. In another embodiment, the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding is present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. See, *e.g.*, Suresh *et al.*, 1986 *Methods in Enzymology*, 121: 210.

In another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. See, *e.g.*, PCT Publication WO 96/27011. An interface may comprise at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of a first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of a second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Any procedure known in the art wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments may be used. See, *e.g.*, Brennan *et al.*, 1985 *Science* 229: 81. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. One embodiment includes the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. See, *e.g.*, Shalaby *et al.*, 1992 *J. Exp. Med.* 175:217-225. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. See, *e.g.*, Kostelny *et al.*, 1992 *J. Immunol.* 148: 1547-1553. The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology is an alternative mechanism for making bispecific antibody fragments. See, *e.g.*, Hollinger *et al.*, 1993 *Proc. Natl. Acad. Sci. USA* 90: 6444-6448. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, *e.g.*, Gruber *et al.*, 1994 *J. Immunol.* 152: 5368.

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. See, *e.g.*, Tutt *et al.*, 1991 *J. Immunol.* 147: 60.

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another

bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

7. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT Publications WO 91/00360 and WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate, methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

8. Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See, *e.g.*, Caron *et al.*, 1992 *J. Exp Med.* 176: 1191-1195; Shopes, 1992 *J. Immunol.*, 148: 2918-2922. Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers. See, *e.g.*, Wolff *et al.* 1993 *Cancer Research*, 53: 2560-2565. Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See, *e.g.*, Stevenson *et al.*, 1989 *Anti-Cancer Drug Design* 3: 219-230.

9. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain

(from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared. See, *e.g.*, Vitetta *et al.*, 1987 *Science*, 238: 1098. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, *e.g.*, PCT Publication WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is in turn conjugated to a cytotoxic agent.

10. Immunoliposomes

The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art. See, *e.g.*, Epstein *et al.*, 1985 *Proc. Natl. Acad. Sci. USA*, 82: 3688; Hwang *et al.*, 1980 *Proc. Natl Acad. Sci. USA*, 77: 4030; and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are also contemplated. See, *e.g.*, U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes via a disulfide-interchange reaction. See, *e.g.* Martin *et al.*, 1982 *J. Biol. Chem.*, 257: 286-288. A chemotherapeutic agent

(such as Doxorubicin) is optionally contained within the liposome. See, *e.g.* Gabizon *et al.*, 1989 *J. National Cancer Inst.*, 81: 1484.

11. Diagnostic Applications of Antibodies

Antibodies directed against a protein of the invention may be used in methods known within the art relating to the localization and/or quantitation of the protein (*e.g.*, for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies against the proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antigen binding domain, are utilized as pharmacologically-active compounds (see below).

An antibody specific for a protein of the invention can be used to isolate the protein by standard techniques, such as immunoaffinity chromatography or immunoprecipitation. Such an antibody can facilitate the purification of the natural protein antigen from cells and of recombinantly produced antigen expressed in host cells. Moreover, such an antibody can be used to detect the antigenic protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the antigenic protein. Antibodies directed against the protein can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

12. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a protein of the invention, as well as other molecules identified by the screening assays disclosed herein, can be administered for the treatment of various disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are

known in the art. See, for example, Remington 1995 In: THE SCIENCE AND PRACTICE OF PHARMACY 19th ed. (Alfonso R. Gennaro, *et al.*, editors) Mack Pub. Co., Easton, Pa; DRUG ABSORPTION ENHANCEMENT: CONCEPTS, POSSIBILITIES, LIMITATIONS, AND TRENDS, Harwood Academic Publishers, Langhorne, Pa., 1994; and PEPTIDE AND PROTEIN DRUG DELIVERY In: ADVANCES IN PARENTERAL SCIENCES, Vol. 4, 1991, M. Dekker, New York.

If the antigenic protein is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*, Marasco *et al.*, 1993 *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893. The formulation herein can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules) or in macroemulsions.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (see, *e.g.*, U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT

™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

13. Antibody Therapeutics

Antibodies of the invention, including polyclonal, monoclonal, humanized and fully human antibodies, may used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology in a subject. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Such an effect may be one of two kinds, depending on the specific nature of the interaction between the given antibody molecule and the target antigen in question. In the first instance, administration of the antibody may abrogate or inhibit the binding of the target with an endogenous ligand to which it naturally binds. In this case, the antibody binds to the target and masks a binding site of the naturally occurring ligand, wherein the ligand serves as an effector molecule. Thus the receptor mediates a signal transduction pathway for which ligand is responsible.

Alternatively, the effect may be one in which the antibody elicits a physiological result by virtue of binding to an effector binding site on the target molecule. In this case the target, a receptor having an endogenous ligand which may be absent or defective in the disease or pathology, binds the antibody as a surrogate effector ligand, initiating a receptor-based signal transduction event by the receptor.

A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target, and in other cases, promotes a physiological response. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

FGF19X Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding FGF19X protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the

design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FGF19X proteins, mutant forms of FGF19X, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FGF19X in prokaryotic or eukaryotic cells. For example, FGF19X can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson 1988 *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc and pET 11d. See, *e.g.*, Amrann *et al.*, 1988 *Gene* 69:301-315; Studier *et al.*, 1990 GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. pp. 60-89.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, *e.g.*, Gottesman 1990 GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. pp. 119-128. Another strategy is to

alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli*. See, *e.g.*, Wada *et al.*, 1992 *Nucl. Acids Res.* 20: 2111-2118. Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the FGF19X expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, *et al.*, 1987 *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, 1982 *Cell* 30:933-943), pJRY88 (Schultz *et al.*, 1987 *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, FGF19X can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* 1983 *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers 1989 *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 and pMT2PC. See, *e.g.*, Seed 1987 *Nature* 329:840; Kaufman *et al.* 1987 *EMBO J* 6: 187-195. When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* 1987 *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton 1988 *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore 1989 *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* 1983 *Cell* 33:729-740; Queen and Baltimore 1983 *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle 1989 *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* 1985 *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No.

4,873,316; European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss 1990 *Science* 249:374-379) and the α-fetoprotein promoter (Campes *et al.* 1989 *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to FGF19X mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see, *e.g.*, Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, FGF19X protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding FGF19X or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) FGF19X protein. Accordingly, the invention further provides methods for producing FGF19X protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding FGF19X has been introduced) in a suitable medium such that FGF19X protein is produced. In another embodiment, the method further comprises isolating FGF19X from the medium or the host cell.

Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FGF19X-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous FGF19X sequences have been introduced into their genome or homologous recombinant animals in which endogenous FGF19X sequences have been altered. Such animals are useful for studying the function and/or activity of FGF19X and for identifying and/or evaluating modulators of FGF19X activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a

transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous FGF19X gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FGF19X-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FGF19X DNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human FGF19X gene, such as a mouse FGF19X gene, can be isolated based on hybridization to the human FGF19X cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the FGF19X transgene to direct expression of FGF19X protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the FGF19X transgene in its genome and/or expression of FGF19X mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding FGF19X can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a FGF19X gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the FGF19X gene. The FGF19X gene can be a human gene (*e.g.*, SEQ ID NO:1), but more preferably, is a non-human homologue of a human FGF19X gene. For example, a mouse homologue of human FGF19X gene of SEQ ID NO:1 can be used to construct a homologous recombination vector suitable for altering an endogenous FGF19X gene in the mouse genome. In one embodiment, the vector is designed

such that, upon homologous recombination, the endogenous FGF19X gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FGF19X gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FGF19X protein). In the homologous recombination vector, the altered portion of the FGF19X gene is flanked at its 5' and 3' ends by additional nucleic acid of the FGF19X gene to allow for homologous recombination to occur between the exogenous FGF19X gene carried by the vector and an endogenous FGF19X gene in an embryonic stem cell. The additional flanking FGF19X nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector used for the homologous recombination. See *e.g.*, Thomas *et al.* 1987 *Cell* 51:503. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced FGF19X gene has homologously recombined with the endogenous FGF19X gene are selected. See *e.g.*, Li *et al.* 1992 *Cell* 69:915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos. WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* 1992 *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* 1991 *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre

recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced by any method known in the art. See, *e.g.*, Wilmut *et al.* 1997 *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

Pharmaceutical Compositions

The FGF19X nucleic acid molecules, FGF19X proteins, and anti-FGF19X antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's PHARMACEUTICAL SCIENCES, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral,

intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a FGF19X protein or anti-FGF19X antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable

solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of

such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art. See, *e.g.*, U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by any of a number of routes, *e.g.*, as described in U.S. Patent Nos. 5,703,055. Delivery can thus also include, *e.g.*, intravenous injection, local administration (see *e.g.*, U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Uses and Methods of the Invention

Various FGFs have been implicated in oncogenic disorders. *In vitro* studies and tumor xenograft experiments (both orthotopic and subcutaneous models) show that FGFs have a role in, for example, malignant melanoma, breast adenocarcinoma, transitional carcinoma of the bladder, small cell lung cancer, prostate adenocarcinoma and malignant gliomas (Ellis and Fidler; 1996 *Eur J Cancer* 32A: 2451-2460; Goustin *et al.* 1986 *Cancer Res* 46: 1015-1029). As shown in EXAMPLE 5, FGF19X is expressed in at least lung carcinomas, colon cancers, prostate cancers, ovarian cancers, and the like. This suggests a potential role of FGF19X is these diseases.

The potential role(s) of FGF19X in tumorigenesis may include autocrine stimulation of tumor growth, hormone independence, angiogenesis, metastatic progression, chemoresistance, radiotherapy resistance, survival in trophic factor limited secondary tissue site microenvironments, and stimulation of tumor cell matrix degradation and tumor cell migration (*i.e.*, tumor invasion).

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) detection assays (*e.g.*, chromosomal mapping, tissue typing, forensic biology), (c) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, in one embodiment, a FGF19X protein of the invention has the ability to bind at least one FGFR.

The isolated nucleic acid molecules of the invention can be used to express FGF19X protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect FGF19X mRNA (*e.g.*, in a biological sample) or a genetic lesion in a FGF19X gene, and to modulate FGF19X activity, as described further below. In addition, the FGF19X proteins can be used to screen drugs or compounds that modulate the FGF19X activity or expression as well as to treat disorders characterized by insufficient or excessive production of FGF19X protein, for example proliferative or differentiative disorders, or production of FGF19X protein forms that have decreased or aberrant activity compared to FGF19X wild type protein. In addition, the anti-FGF19X antibodies of the invention can be used to detect and isolate FGF19X proteins and modulate FGF19X activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FGF19X proteins or have a stimulatory or inhibitory effect on, for example, FGF19X expression or FGF19X activity.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a FGF19X protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase

libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. See, *e.g.*, Lam 1997 *Anticancer Drug Des* 12:145.

Examples of methods for the synthesis of molecular libraries can be found in the art. See, *e.g.*, DeWitt *et al.* 1993 *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* 1994 *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* 1994 *J Med Chem* 37:2678; Cho *et al.* 1993 *Science* 261:1303; Carrell *et al.* 1994 *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* 1994 *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* 1994 *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten 1992 *Biotechniques* 13:412-421), or on beads (Lam 1991 *Nature* 354:82-84), on chips (Fodor 1993 *Nature* 364: 555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* 1992 *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith 1990 *Science* 249:386-390; Devlin 1990 *Science* 249:404-406; Cwirla *et al.* 1990 *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici 1991 *J Mol Biol* 222:301-310; Ladner above.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of FGF19X protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a FGF19X protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FGF19X protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FGF19X protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of FGF19X protein, or a biologically active portion thereof, on the cell surface with a known compound which binds FGF19X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FGF19X protein, wherein determining the ability of the test compound to interact with a FGF19X

protein comprises determining the ability of the test compound to preferentially bind to FGF19X or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of FGF19X protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FGF19X protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of FGF19X or a biologically active portion thereof can be accomplished, for example, by determining the ability of the FGF19X protein to bind to or interact with a FGF19X target molecule. As used herein, a "target molecule" is a molecule with which a FGF19X protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a FGF19X interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A FGF19X target molecule can be a non-FGF19X molecule or a FGF19X protein or polypeptide of the present invention. In one embodiment, a FGF19X target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.*, a signal generated by binding of a compound to a membrane-bound FGF19X molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FGF19X.

Determining the ability of the FGF19X protein to bind to or interact with a FGF19X target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FGF19X protein to bind to or interact with a FGF19X target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a FGF19X-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a FGF19X protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the FGF19X protein or

biologically active portion thereof. Binding of the test compound to the FGF19X protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the FGF19X protein or biologically active portion thereof with a known compound which binds FGF19X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FGF19X protein, wherein determining the ability of the test compound to interact with a FGF19X protein comprises determining the ability of the test compound to preferentially bind to FGF19X or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting FGF19X protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FGF19X protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of FGF19X can be accomplished, for example, by determining the ability of the FGF19X protein to bind to a FGF19X target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FGF19X can be accomplished by determining the ability of the FGF19X protein further modulate a FGF19X target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the FGF19X protein or biologically active portion thereof with a known compound which binds FGF19X to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FGF19X protein, wherein determining the ability of the test compound to interact with a FGF19X protein comprises determining the ability of the FGF19X protein to preferentially bind to or modulate the activity of a FGF19X target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of FGF19X. In the case of cell-free assays comprising the membrane-bound form of FGF19X, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of FGF19X is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, octanoyl-N-methylglucamide, n-dodecylglucoside, n-dodecylmaltoside, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl-N,N-dimethyl-3-ammonio-1-propane

sulfonate, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either FGF19X or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FGF19X, or interaction of FGF19X with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FGF19X fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or FGF19X protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of FGF19X binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either FGF19X or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FGF19X or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FGF19X or target molecules, but which do not interfere with binding of the FGF19X protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FGF19X trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FGF19X or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FGF19X or target molecule.

In another embodiment, modulators of FGF19X expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FGF19X mRNA or protein in the cell is determined. The level of expression of FGF19X mRNA or protein in the presence of the candidate compound is compared to the level of expression of FGF19X mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FGF19X expression based on this comparison. For example, when expression of FGF19X mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FGF19X mRNA or protein expression. Alternatively, when expression of FGF19X mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FGF19X mRNA or protein expression. The level of FGF19X mRNA or protein expression in the cells can be determined by methods described herein for detecting FGF19X mRNA or protein.

In yet another aspect of the invention, the FGF19X proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* 1993 *Cell* 72:223-232; Madura *et al.* 1993 *J Biol Chem* 268:12046-12054; Bartel *et al.* 1993 *Biotechniques* 14:920-924; Iwabuchi *et al.* 1993 *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with FGF19X ("FGF19X-binding proteins" or "FGF19X-bp") and modulate FGF19X activity. Such FGF19X-binding proteins are also likely to be involved in the propagation of signals by the FGF19X proteins as, for example, upstream or downstream elements of the FGF19X pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FGF19X is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a FGF19X-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be

detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FGF19X.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the FGF19X cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample.

The FGF19X sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms"). See, *e.g.*, U.S. Pat. No. 5,272,057.

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FGF19X sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The FGF19X sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because

greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, as described above, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining FGF19X protein and/or nucleic acid expression as well as FGF19X activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant FGF19X expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with FGF19X protein, nucleic acid expression or activity. For example, mutations in a FGF19X gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with FGF19X protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining FGF19X protein, nucleic acid expression or FGF19X activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to as "pharmacogenomics" herein). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FGF19X in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

Fibroblast growth factors FGF-1 through FGF-9 generally promote cell proliferation in cells carrying the particular growth factor receptor. Examples of FGF growth promotion include epithelial cells, such as fibroblasts and keratinocytes, in the anterior eye after surgery. Other conditions in which proliferation of cells plays a role include tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi's sarcoma and rheumatoid arthritis.

FGF19X may be used in the method of the invention for detecting its corresponding fibroblast growth factor receptor (FGF19R) in a sample or tissue. The method comprises contacting the sample or tissue with FGF19X, allowing formation of receptor-ligand pairs, and detecting any FGF19R: FGF19X pairs. Compositions containing FGF19X can be used to increase FGF19R activity, for example to stimulate cartilage or bone repair. Compositions containing FGF19X antagonists or FGF19X binding agents (*e.g.* anti- FGF19X antibodies) can be used to treat diseases caused by an excess of FGF19X or overactivity of FGFRCX, especially multiple or solitary hereditary exostosis, hallux valgus deformity, achondroplasia, synovial chondromatosis and endochondromas.

FGF19X can also be used to stimulates fibroblasts (for accelerating healing of burns, wounds, ulcers, etc), megakaryocytes (to increase the number of platelets), hematopoietic cells, immune system cells, and vascular smooth muscle cells. FGF19X is also expected to have osteogenesis-promoting activity, and can be used for treating bone fractures and osteoporosis. Assay of FGF19X polypeptide or nucleic acid moieties may be useful in diagnosis of cerebral tumors, and antibodies against could be used to treat such tumors. It can also be used as a reagent for stimulating growth of cultured cells. An anticipated dosage is 1ng-0.1mg/kg/day, though treatment may vary depending on the type or severity of the disorder being treated. FGF19X polypeptides may be used as platelet increasing agents, osteogenesis promoting agents or for treating cerebral nervous diseases or hepatopathy such as hepatic cirrhosis. They can also be used to treat cancer when used alongside an anticancer agent. Antibodies directed against the FGF19X polypeptide, or fragments, derivatives, or analogs thereof, can be used for detecting or determining a biological activity of a FGF19X polypeptide or for purifying a FGF19X polypeptide. Those antibodies that also neutralize the cell growth activity of FGF19X can be used as anticancer agents.

Many, if not all, homologous proteins are known in the art to have closely related or identical functions. See, *e.g.*, Lewin, "Chapter 21: Structural Genes Belong to Families" In: GENES II, 1985, John Wiley and Sons, Inc., New York. The FGF19X polypeptide closely

resembles the Xenopus XFGF-20 protein, which was shown previously to be specifically expressed in highly proliferative tissues (see, *e.g.*, Koga *et al.*, above). Therefore, it is presumed that FGF19X would also modulate cellular activity in highly proliferative tissues. FGF19X may thus be particularly useful in diagnosing proliferative disorders and in stimulating the growth of cells and tissues in order to overcome pathological states in which such growth has been suppressed or inhibited. Oligonucleotides corresponding to any one portion of the FGF19X nucleic acids of SEQ ID NO:1 may be used to detect the expression of a FGF19X-like gene. The proteins of the invention may be used to stimulate production of antibodies specifically binding the proteins. Such antibodies may be used in immunodiagnostic procedures to detect the occurrence of the protein in a sample. The proteins of the invention may be used to stimulate cell growth and cell proliferation in conditions in which such growth would be favorable. An example would be to counteract toxic side effects of chemotherapeutic agents on, for example, hematopoiesis and platelet formation, linings of the gastrointestinal tract, and hair follicles. They may also be used to stimulate new cell growth in neurological disorders including, for example, Alzheimer's disease. Alternatively, antagonistic treatments may be administered in which an antibody specifically binding the FGF19X -like proteins of the invention would abrogate the specific growth-inducing effects of the proteins. Such antibodies may be useful, for example, in the treatment of proliferative disorders including various tumors and benign hyperplasias.

An exemplary method for detecting the presence or absence of FGF19X in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting FGF19X protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes FGF19X protein such that the presence of FGF19X is detected in the biological sample. An agent for detecting FGF19X mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to FGF19X mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FGF19X nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to FGF19X mRNA or genomic DNA, as described above. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting FGF19X protein is an antibody capable of binding to FGF19X protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass

direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect FGF19X mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of FGF19X mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of FGF19X protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of FGF19X genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of FGF19X protein include introducing into a subject a labeled anti-FGF19X antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting FGF19X protein, mRNA, or genomic DNA, such that the presence of FGF19X protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of FGF19X protein, mRNA or genomic DNA in the control sample with the presence of FGF19X protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of FGF19X in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting FGF19X protein or mRNA in a biological sample; means for determining the amount of FGF19X in the sample; and means for comparing the amount of FGF19X in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect FGF19X protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FGF19X expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with FGF19X protein, nucleic acid expression or activity in, *e.g.*, proliferative or differentiative disorders such as hyperplasias, carcinogenesis, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, or rheumatoid arthritis, etc. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant FGF19X expression or activity in which a test sample is obtained from a subject and FGF19X protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of FGF19X protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FGF19X expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FGF19X expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as a proliferative disorder, differentiative disorder, tumorigenic disorders, etc. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FGF19X expression or activity in which a test sample is obtained and FGF19X protein or nucleic acid is detected (*e.g.*, wherein the presence of FGF19X protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FGF19X expression or activity.)

The methods of the invention can also be used to detect genetic lesions in a FGF19X gene, thereby determining if a subject with the lesioned gene is at risk for, or suffers from, a proliferative disorder, differentiative disorder, tumorigenic disorder, etc. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting

the integrity of a gene encoding a FGF19X-protein, or the mis-expression of the FGF19X gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from a FGF19X gene; (2) an addition of one or more nucleotides to a FGF19X gene; (3) a substitution of one or more nucleotides of a FGF19X gene, (4) a chromosomal rearrangement of a FGF19X gene; (5) an alteration in the level of a messenger RNA transcript of a FGF19X gene, (6) aberrant modification of a FGF19X gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a FGF19X gene, (8) a non-wild type level of a FGF19X-protein, (9) allelic loss of a FGF19X gene, and (10) inappropriate post-translational modification of a FGF19X-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a FGF19X gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* 1988 *Science* 241:1077-1080; and Nakazawa *et al.* 1994 *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the FGF19X-gene (see *e.g.*, Abravaya *et al.* 1995 *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a FGF19X gene under conditions such that hybridization and amplification of the FGF19X gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (see *e.g.*, Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (see *e.g.*, Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (see *e.g.*, Lizardi *et al*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially

useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a FGF19X gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site. See *e.g.*, U.S. Pat. No. 5,493,531.

In other embodiments, genetic mutations in FGF19X can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes. See, *e.g.* Cronin *et al.* 1996 *Human Mutation* 7: 244-255; Kozal *et al.* 1996 *Nature Medicine* 2: 753-759. For example, genetic mutations in FGF19X can be identified in two dimensional arrays containing light-generated DNA probes. See, *e.g.* Cronin *et al.* 1996, above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FGF19X gene and detect mutations by comparing the sequence of the sample FGF19X with the corresponding wild-type (control) sequence. Examples of sequencing reactions include, but are not limited to, those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays, including sequencing by mass spectrometry. See, *e.g.*, PCT Publ. No. WO 94/16101; Naeve *et al.*, 1995 *Biotechniques* 19:448; Cohen *et al.* 1996 *Adv Chromatogr* 36:127-162; and Griffin *et al.* 1993 *Appl Biochem Biotechnol* 38:147-159.

Other methods for detecting mutations in the FGF19X gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or

RNA/DNA heteroduplexes. See, *e.g*, Myers *et al.* 1985 *Science* 230:1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FGF19X sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* 1988 *PNAS USA* 85:4397; Saleeba *et al* 1992 *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FGF19X cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. See, *e.g.* Hsu *et al.* 1994 *Carcinogenesis* 15:1657-1662. According to an exemplary embodiment, a probe based on a FGF19X sequence, *e.g.*, a wild-type FGF19X sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, *e.g.*, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FGF19X genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. See, *e.g.*, Orita *et al.* 1989 *Proc Natl Acad Sci USA*: 86: 2766; Cotton 1993 *Mutat Res* 285:125-144; Hayashi 1992 *Genet Anal Tech Appl* 9:73-79. Single-stranded DNA fragments of sample and control FGF19X nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA, rather than DNA, in which the

secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. See, *e.g.*, Keen *et al.* 1991 *Trends Genet* 7: 5.

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). See, *e.g.*, Myers *et al* 1985 *Nature* 313:495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. See, *e.g.*, Rosenbaum and Reissner 1987 *Biophys Chem* 265:12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. See, *e.g.*, Saiki *et al.* 1986 *Nature* 324:163); Saiki *et al.* 1989 *Proc Natl Acad. Sci USA* 86:6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (see *e.g.*, Gibbs *et al.* 1989 *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (see *e.g.*, Prossner 1993 *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. See, *e.g.*, Gasparini *et al* 1992 *Mol Cell Probes* 6:1. It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification. See, *e.g.*, Barany 1991 *Proc Natl Acad Sci USA* 88:189. In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a FGF19X gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which FGF19X is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on FGF19X activity (*e.g.*, FGF19X gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (*e.g.*, neurological, cancer-related or gestational disorders) associated with aberrant FGF19X activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of FGF19X protein, expression of FGF19X nucleic acid, or mutation content of FGF19X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996, *Clin Exp Pharmacol Physiol*, 23:983-985 and Linder, 1997, *Clin Chem*, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical

complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of FGF19X protein, expression of FGF19X nucleic acid, or mutation content of FGF19X genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a FGF19X modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring Clinical Efficacy

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FGF19X (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied in basic drug screening and in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FGF19X gene expression, protein levels, or upregulate FGF19X activity, can be monitored in clinical trials of subjects exhibiting decreased FGF19X gene expression, protein levels, or

downregulated FGF19X activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FGF19X gene expression, protein levels, or downregulate FGF19X activity, can be monitored in clinical trials of subjects exhibiting increased FGF19X gene expression, protein levels, or upregulated FGF19X activity. In such clinical trials, the expression or activity of FGF19X and, preferably, other genes that have been implicated in, for example, a proliferative or neurological disorder, can be used as a "read out" or marker of the responsiveness of a particular cell.

For example, genes, including FGF19X, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates FGF19X activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of FGF19X and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of FGF19X or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, nucleic acid, peptidomimetic, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of a FGF19X protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the FGF19X protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the FGF19X protein, mRNA, or genomic DNA in the pre-administration sample with the FGF19X protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of FGF19X to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased

administration of the agent may be desirable to decrease expression or activity of FGF19X to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FGF19X expression or activity.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (*i*) a FGF19X polypeptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to a FGF19X peptide; (*iii*) nucleic acids encoding a FGF19X peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to a FGF19X peptide) that are utilized to "knockout" endogenous function of a FGF19X peptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between a FGF19X peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a FGF19X peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a FGF19X peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant FGF19X expression or activity, by administering to the subject an agent that modulates FGF19X expression or at least one FGF19X activity. Subjects at risk for a disease that is caused or contributed to by aberrant FGF19X expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FGF19X aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of FGF19X aberrancy, for example, a FGF19X agonist or FGF19X antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

Another aspect of the invention pertains to methods of modulating FGF19X expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of FGF19X protein activity associated with the cell. An agent that modulates FGF19X protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a FGF19X protein, a peptide, a FGF19X peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more FGF19X protein activity. Examples of such stimulatory agents include active FGF19X protein and a nucleic acid molecule encoding FGF19X that has been introduced into the cell. In another embodiment, the agent inhibits one or more FGF19X protein activity. Examples of such inhibitory agents include antisense FGF19X nucleic acid molecules and anti-FGF19X antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a FGF19X protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) FGF19X expression or activity. In another embodiment, the method involves administering a FGF19X protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FGF19X expression or activity.

The invention will be further illustrated in the following examples, which do not limit the scope of the claims.

EXAMPLES

Example 1. Molecular Cloning of FGF19X

FGF19X, a novel FGF-19 homolog, was identified from human genomic DNA. The FGF19X cDNA (SEQ ID NO:1) was cloned. The predicted open reading frame of the FGF19X cDNA encodes a 153 residue FGF19X polypeptide (SEQ ID NO:2). A predicted signal peptide sequence is expected to be cleaved between residues 28 and 29, as described above.

The following oligonucleotide primers were designed to PCR amplify the mature form of FGF19X from residue 29 to 153 of SEQ ID NO:1.

FGF19X MAT Forward: 5' GGATCC CAC CCC ATC CCT GAC TCC AGT CCT CTC 3' (SEQ ID NO:4)

FGF19X MAT Reverse: 5' CTC GAG GAG CCT CCT CTG TAA CCC TGG CAG GTG 3' (SEQ ID NO:5).

For cloning purposes, the forward primer includes an in-frame BamHI restriction site, and the reverse primer contains an in-frame XhoI restriction site.

Two separate PCR reactions were set up using, respectively, 5 ng human pituitary cDNA template, and a total of 5 ng cDNA combined from equal amounts of cDNAs derived from human testis, human fetal brain, human mammary tissue and human skeletal muscle. The reaction mixtures contained 1 microM of each of the FGF19X MAT Forward and FGF19X MAT Reverse primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50 X Advantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliters.

The following PCR reaction conditions were used:

a) 96°C 3 minutes;

b) 96°C 30 seconds denaturation;

c) 70°C 30 seconds, primer annealing. This temperature was gradually decreased by 1°C per cycle;

d) 72°C 1 minute extension;

Repeat steps (b)-(d) 10 times;

e) 96°C 30 seconds denaturation;

f) 60°C 30 seconds annealing;

g) 72°C 1 minute extension;

Repeat steps (e)-(g) 25 times; and

h) 72°C 5 minutes final extension.

The expected amplified product of 375 bp was detected by agarose gel electrophoresis in both samples. The fragments were purified from agarose gel and ligated to pCR2.1 vector (Invitrogen, Carlsbad, CA). The cloned inserts were sequenced and verified as an open reading frame encoding the predicted mature form of FGF19X. The construct is called pCR2.1-FGF19X. The sequence obtained in pCR2.1-FGF19X is identical to the sequence predicted from SEQ ID NO:1.

Example 2. Preparation of expression vector pCEP4/Sec

The following oligonucleotide primers were prepared in order to amplify a fragment from the pcDNA3.1-V5His expression vector (Invitrogen, Carlsbad, CA).

pSec-V5-His Forward CTCGTCCTCGAGGGTAAGCCTATCCCTAAC (SEQ ID NO:6)

pSec-V5-His Reverse CTCGTCGGGCCCCTGATCAGCGGGTTTAAAC (SEQ ID NO:7)

The PCR product was digested with XhoI and ApaI and ligated into the XhoI/ApaI digested pSecTag2 B vector harboring an Ig kappa leader sequence (Invitrogen, Carlsbad CA). The correct structure of the resulting vector, pSecV5His, was verified by DNA sequence analysis. The vector pSecV5His was digested with PmeI and NheI, and the PmeI-NheI fragment was ligated into the BamHI/Klenow and NheI treated vector pCEP4 (Invitrogen, Carlsbad, CA). The resulting vector was named pCEP4/Sec.

Example 3. Expression of FGF19X in recombinant *E. coli.*

The vector pRSETA (InVitrogen Inc., Carlsbad, CA) was digested with XhoI and NcoI restriction enzymes. The following oligonucleotide linkers were annealed at 37°C and ligated into the XhoI-NcoI treated pRSETA.

Linker 1: CATGGTCAGCCTAC (SEQ ID NO:8) and

Linker 2: TCGAGTAGGCTGAC (SEQ ID NO:9)

The resulting vector was confirmed by restriction analysis and sequencing and was named pETMY. A 0.4 kb BamHI-XhoI fragment containing the mature portion of the hFGF19X sequence was isolated from pCR2.1-FGF19X (EXAMPLE 1). This fragment was ligated into the pETMY vector that had been digested with BamHI and XhoI restriction enzymes. The resulting expression vector including the mature FGF19X sequence is named pETMY-FGF19X. In this vector, hFGF19X was fused to the 6xHis tag and T7 epitope at its N-terminus. The plasmid pETMY-FGF19X was then transformed into the *E. coli* expression host

BL21 (DE3, pLys) (Novagen, Madison, WI) and the induction of protein expression was carried out according to the manufacturer's instructions. After induction, the cells were harvested, and proteins were analyzed by Western blotting using anti-HisGly antibody (Invitrogen, Carlsbad, CA). The hFGF19X was expressed as a protein having an apparent molecular weight of about 23 kDa in *E. coli* cells, as shown in FIG. 1.

Example 4. Expression of hFGF19X in human embryonic kidney 293 cells.

The 0.4 kb BamHI-XhoI fragment containing the hFGF19X sequence was isolated from pCR2.1-FGF19X (EXAMPLE 1) and subcloned into BamHI-XhoI digested pCEP4/Sec to generate expression vector pCEP4/Sec-FGF19X. The pCEP4/Sec-FGF19X vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's instructions (Gibco/BRL). The cell pellet and supernatant were harvested 72 hours after transfection and examined for hFGF19X expression by Western blotting after SDS-PAGE run under reducing conditions with an anti-V5 antibody. The hFGF19X was expressed as a protein having an apparent molecular weight of about 23 kDa secreted by 293 cells, as shown in FIG. 2.

Example 5. Tissue Profile of Expression of FGF19X

The quantitative expression of various clones was assessed in 41 normal and 55 tumor samples (see Tables 4 below) by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System

First, 96 RNA samples were normalized to beta-actin and GAPDH RNA (~50 ng total or ~1 ng polyA+), and were converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; cat # N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 microliter (µl) reactions and incubated for 30 min. at 48°C. The cDNA (5 µl) was then transferred to a separate plate for the TAQMAN® reaction using beta-actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; cat. no's 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; catalog no. 4304447) according to the manufacturer's protocol. Reactions were performed in 25 µl reactions using the following parameters: 2 min. at 50°C; 10 min. at 95°C; then 40 cycles of 15 sec. at 95°C and 1 min. at 60°C. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between two samples being represented as 2 to the power of delta CT ($2^{\Delta CT}$). The average CT values

obtained for ß-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their beta-actin /GAPDH average CT values.

Normalized RNA (5 µl) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; cat. # 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) using the sequence of FGF19X as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM; primer melting temperature (T_m) range = 58°-60° C; primer optimal Tm = 59° C; maximum primer difference = 2° C; probe does not have a 5' G; probe T_m must be 10° C greater than primer T_m; and amplicon size of 75 bp to 100 bp. The probes and primers selected (Ab78 series, see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double HPLC purified to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were 900 nM each for forward (F) and reverse (R) primers and 200 nM for probe (P). The primers and probe used were as follows:

Ab78 (F): 5'-GACCAGCCAGCACAGAAACC-3' (SEQ ID NO:10)

Ab78 (R): 5'-GGACCCGAGCCATTGATG-3' (SEQ ID NO:11)

Ab78 (P): FAM-5'-TCCTGAGTGCTCGAACCCGGTCTC-3'-TAMRA (SEQ ID NO:12)

PCR conditions used were as follow: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (FGF19X-specific and another gene-specific probe multiplexed with the FGF19X probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM $MgCl_2$, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/µl RNase inhibitor, and 0.25 U/µl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification. PCR cycles were as follows: 95° C for 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

As shown in TABLE 4, FGF19X is highly expressed in small cell lung cancers. FGF19X expression is also seen in colon cancer, fetal liver, prostate cancer, myometria,

ovarian cancer, adipose cells, gliocytes (CNS), squamous cell lung cancers, ascending colon, and other tissues.

TABLE 4: FGF19X TISSUE EXPRESSION RESULTS

well #	TISSUE	Relative Expression (%)
1	Endothelial cells	0.00
2	Endothelial cells (treated)	0.00
3	Pancreas	0.81
4	Pancreatic cancer CAPAN 2	0.00
5	Adipose	10.88
6	Adrenal gland	0.37
7	Thyroid	0.00
8	Salavary gland	0.00
9	Pituitary gland	0.00
10	Brain (fetal)	0.00
11	Brain (whole)	0.00
12	Brain (amygdala)	0.00
13	Brain (cerebellum)	0.79
14	Brain (hippocampus)	0.00
15	Brain (hypothalamus)	0.00
16	Brain (substantia nigra)	0.59
17	Brain (thalamus)	0.00
18	Spinal cord	0.00
19	CNS cancer (glio/astro) U87-MG	0.00
20	CNS cancer (glio/astro) U-118-MG	0.00
21	CNS cancer (astro) SW1783	0.24
22	CNS cancer* (neuro; met) SK-N-AS	0.47
23	CNS cancer (astro) SF-539	0.00
24	CNS cancer (astro) SNB-75	0.00
25	CNS cancer (glio) SNB-19	1.95
26	CNS cancer (glio) U251	0.00
27	CNS cancer (glio) SF-295	8.30
28	Heart	0.00
29	Skeletal muscle	0.89
30	Bone marrow	0.00
31	Thymus	0.00
32	Spleen	0.00
33	Lymph node	0.56
34	Colon (ascending)	8.72
35	Stomach	1.11
36	Small intestine	0.00
37	Colon cancer SW480	0.79

38	Colon cancer* (SW480 met)SW620	2.66
39	Colon cancer HT29	0.45
40	Colon cancer HCT-116	0.00
41	Colon cancer CaCo-2	0.87
42	Colon cancer HCT-15	35.85
43	Colon cancer HCC-2998	0.00
44	Gastric cancer* (liver met) NCI-N87	0.56
45	Bladder	1.99
46	Trachea	0.00
47	Kidney	0.00
48	Kidney (fetal)	0.00
49	Renal cancer 786-0	0.00
50	Renal cancer A498	0.52
51	Renal cancer RXF 393	0.00
52	Renal cancer ACHN	0.15
53	Renal cancer UO-31	0.00
54	Renal cancer TK-10	0.00
55	Liver	1.71
56	Liver (fetal)	30.99
57	Liver cancer (hepatoblast) HepG2	0.52
58	Lung	0.00
59	Lung (fetal)	0.22
60	Lung cancer (small cell) LX-1	2.50
61	Lung cancer (small cell) NCI-H69	88.27
62	Lung cancer (small cell variant) SHP-77	100.00
63	Lung cancer (large cell) NCI-H460	7.97
64	Lung cancer (non-small cell) A549	2.13
65	Lung cancer (non-small cell) NCI-H23	0.27
66	Lung cancer (non-small cell) HOP-62	0.00
67	Lung cancer (non-small cell) NCI-H522	0.00
68	Lung cancer (squamous cell) SW 900	0.53
69	Lung cancer (squamous cell) NCI-H596	6.75
70	Mammary gland	0.27
71	Breast cancer* (plural. effusion) MCF-7	0.27
72	Breast cancer* (plural effusion) MDA-MB-231	0.00
73	Breast cancer* (plural effusion) T47D	3.04
74	Breast cancer BT-549	2.16
75	Breast cancer MDA-N	0.68
76	Ovary	0.00
77	Ovarian cancer OVCAR-3	0.14
78	Ovarian cancer OVCAR-4	0.75
79	Ovarian cancer OVCAR-5	4.54
80	Ovarian cancer OVCAR-8	0.24

81	Ovarian cancer IGROV-1	0.14
82	Ovarian cancer* (ascites) SK-OV-3	0.00
83	Myometrium	9.02
84	Uterus	0.50
85	Plancenta	0.00
86	Prostate	0.00
87	Prostate cancer* (bone met)PC-3	4.84
88	Testis	3.08
89	Melanoma Hs688(A).T	0.00
90	Melanoma* (met) Hs688(B).T	0.81
91	Melanoma UACC-62	0.16
92	Melanoma M14	0.43
93	Melanoma LOX IMVI	0.00
94	Melanoma* (met) SK-MEL-5	1.01
95	Melanoma SK-MEL-28	0.45
96	Melanoma UACC-257	0.45

KEY: glio. = gliocyte; astro. = astrocyte; neuro. = neurocyte; met. = metastatic; CNS = central nervous system

EQUIVALENTS

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that particular novel compositions and methods involving nucleic acids, polypeptides, antibodies, detection and treatment have been described. Although these particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made as a matter of routine for a person of ordinary skill in the art to the invention without departing from the spirit and scope of the invention as defined by the claims. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated nucleic acid molecule encoding FGF19X, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 85% identical to SEQ ID NO:2, or the complement of said nucleic acid molecule.

2. The nucleic acid molecule of claim 1, wherein said nucleotide sequence encodes a polypeptide of SEQ ID NO:2, or the complement of said nucleic acid molecule.

3. The nucleic acid molecule of claim 1, said molecule encoding the human FGF19X of SEQ ID NO:1, or the complement of said nucleic acid molecule.

4. The isolated nucleic acid molecule of claim 1, said molecule hybridizing under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule comprising the sequence of nucleotides of SEQ ID NO:1, or the complement of said nucleic acid molecule.

5. The isolated nucleic acid molecule of claim 1, said molecule encoding a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or an amino acid sequence comprising one or more conservative substitutions in the amino acid sequence of SEQ ID NO:2.

6. An oligonucleotide of the nucleic acid molecule of claim 1, said nucleic acid molecule less than 100 nucleotides in length and comprising at lease 6 contiguous nucleotides of SEQ ID NO:1, or a complement thereof.

7. A nucleic acid vector comprising the nucleic acid molecule of claim 1.

8. The nucleic acid vector of claim 7, wherein said vector is an expression vector.

9. The vector of claim 7, further comprising a regulatory element operably linked to said nucleic acid molecule.

10. A host cell comprising the isolated nucleic acid molecule of claim 1.

11. An isolated polypeptide at least 80% identical to a polypeptide selected from the group consisting of:

a) a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

b) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NO:2;

c) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

d) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

e) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; and

f) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule of SEQ ID NO:1 under stringent conditions.

12. The polypeptide of claim 11, wherein the polypeptide, or fragment thereof, has an activity selected from the group consisting of:

a) a fibroblast growth factor-like activity;

b) a cell proliferative activity;

c) a tumorigenic activity; and

d) a neuroprotective-like activity.

13. An antibody that selectively binds to the polypeptide of claim 11, and fragments, homologs, analogs, and derivatives of said antibody.

14. A method of producing the polypeptide of claim 11, said method comprising the step of culturing the host cell of claim 10 under conditions in which the nucleic acid molecule is expressed.

15. A method of detecting the presence of the polypeptide of claim 11 in a sample, comprising contacting the sample with a compound that selectively binds to the polypeptide of claim 11 and determining whether the compound bound to the polypeptide of claim 11 is present in the sample.

16. A method of detecting the presence of a nucleic acid molecule of claim 1 in a sample, the method comprising contacting the sample with a nucleic acid probe or primer that selectively binds to the nucleic acid molecule and determining whether the nucleic acid probe or primer bound to the nucleic acid molecule of claim 1 is present in the sample.

17. A method for modulating the activity of the polypeptide of claim 11, the method comprising contacting a cell sample comprising the polypeptide of claim 11 with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

18. A method of treating or preventing a disorder, said disorder selected from a proliferative disorder, a differentiative disorder, and a tumorigenic disorder, said method comprising administering to a subject in which such treatment or prevention is desired an amount of a therapeutic selected from the group consisting of:

a) an isolated nucleic acid molecule encoding FGF19X, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 85% identical to SEQ ID NO:2, or the complement of said nucleic acid molecule;

b) an isolated polypeptide at least 80% identical to a polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

ii) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NO:2;

iii) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

iv) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

v) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; and

vi) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule of SEQ ID NO:1 under stringent conditions; and

c) an antibody that selectively binds to the polypeptide of step (b), and fragments, homologs, analogs, and derivatives of said antibody;

wherein said therapeutic is administered in an amount sufficient to treat or prevent any one of a proliferative disorder, a differentiative disorder, and a tumorigenic disorder in said subject.

19. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a therapeutic selected from the group consisting of:

a) an isolated nucleic acid molecule encoding FGF19X, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 85% identical to SEQ ID NO:2, or the complement of said nucleic acid molecule;

b) an isolated polypeptide at least 80% identical to a polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

ii) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NO:2;

iii) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

iv) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

v) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; and

vi) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule of SEQ ID NO:1 under stringent conditions; and

c) an antibody that selectively binds to the polypeptide of step (b), and fragments, homologs, analogs, and derivatives of said antibody;

and a pharmaceutically acceptable carrier.

20. A kit comprising in one or more containers, a therapeutically or prophylactically effective amount of the pharmaceutical composition of claim 19.

21. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a proliferative disorder, a differentiative disorder, and a tumorigenic disorder disorder, wherein said therapeutic is selected from the group consisting of:

a) an isolated nucleic acid molecule encoding FGF19X, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 85% identical to SEQ ID NO:2, or the complement of said nucleic acid molecule;

b) an isolated polypeptide at least 80% identical to a polypeptide selected from the group consisting of:

i) a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

ii) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NO:2;

iii) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

iv) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2;

v) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; and

vi) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NO:2; wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule of SEQ ID NO:1 under stringent conditions; and

c) an antibody that selectively binds to the polypeptide of step (b), and fragments, homologs, analogs, and derivatives of said antibody.

22. A method for screening for a modulator of activity or of latency or predisposition to any one of a proliferative disorder, a differentiative disorder, and a tumorigenic disorder, said method comprising:

a) administering a test compound to a test animal at increased risk for any one of a proliferative disorder, a differentiative disorder, and a tumorigenic disorder, wherein said test animal recombinantly expresses a FGF19X protein;

b) measuring expression the activity of said protein in said test animal;

c) measuring the activity of said protein in a control animal that recombinantly expresses said protein and is not at increased risk for any one of a proliferative disorder, a differentiative disorder, and a tumorigenic disorder; and

d) comparing expression of said protein in said test animal and said control animal, wherein a change in the activity of said protein in said test animal relative to said control animal indicates the test compound is a modulator of latency of any one of a proliferative disorder, a differentiative disorder, and a tumorigenic disorder.

23. The method of claim 22, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

24. A method for determining the presence of or predisposition to a disease associated with altered levels of a FGF19X polypeptide of claim 11, the method comprising:

a) measuring the amount of the polypeptide in a sample from the mammalian subject; and

b) comparing the amount of said polypeptide in step (a) to the amount of the polypeptide present in a control sample,

wherein an alteration in the level of the polypeptide in step (a) as compared to the control sample indicates a disease condition.

25. A method for determining the presence of or predisposition to a disease associated with altered levels of a FGF19X nucleic acid of claim 1, the method comprising:

a) measuring the amount of the nucleic acid in a sample from the mammalian subject; and

b) comparing the amount of said nucleic acid in step (a) to the amount of the nucleic acid present in a control sample,

wherein an alteration in the level of the nucleic acid in step (a) as compared to the control sample indicates a disease condition.

26. A method of treating a pathological state in a mammal, the method comprising administering to the subject a polypeptide to a subject in an amount to alleviate the pathological condition, wherein the polypeptide a polypeptide having an amino acid sequence at least 85% identical to a polypeptide with an amino acid sequence of SEQ ID NO:2, or a biologically active fragment thereof.

27. A method of treating a pathological state in a mammal, the method comprising administering to the subject the antibody of claim 13 in an amount sufficient to alleviate the pathological condition.

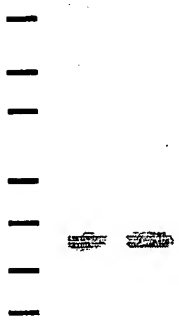

FIG. 1

FIG. 2

INTERNATIONAL SEARCH REPORT

Internal application No
PCT/US 00/24863

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/50 C07K16/22 G01N33/53 A61K38/18
A61K31/70 A61K39/395 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NISHIMURA ET AL: "Structure and expression of a novel human FGF, FGF-19, expressed in fetal brain" BIOCHIMICA ET BIOPHYSICA ACTA,AMSTERDAM,NL, vol. 1444, 18 January 1999 (1999-01-18), pages 148-151, XP002099435 ISSN: 0006-3002 the whole document ---	1,6-27
X	WO 97 15662 A (CHIRON CORP ;RIBOZYME PHARM INC (US)) 1 May 1997 (1997-05-01) page 184 --- -/--	6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 2001	09/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Smalt, R

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INTERNATIONAL SEARCH REPORT

Interr Application No
PCT/US 00/24863

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

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Intern: ıl Application No
PCT/US 00/24863

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 0859837 A	26-08-1998
		JP 2000504925 T	25-04-2000